Acidovorax citrulli is the causative agent of bacterial fruit blotch (BFB) of watermelon [1]. Due to its economic importance, A. citrulli is regarded as a bacterium with plant quarantine significance in U.S., China and Europe. Current available detection methods, such as dilution plating on semi-selective media, seedling grow-out, direct PCR, real-time fluorescent PCR [2,3], have their limitations in that these methods either are time-consuming or require specific equipment. Hence, rapid, sensitive and affordable diagnostic method suitable for on-site detection is urgently needed.

Cross-priming amplification (CPA) is an isothermal DNA amplification system developed by Ustar Biotechnologies Co., Ltd., China. CPA is a class of isothermal amplification reactions that is carried out by a strand displacement DNA polymerase and does not require an initial denaturation step or addition of a nicking enzyme [4]. The detection of amplified products is visualized on a lateral flow strip housed in an enclosed, sealed plastic device to prevent the leakage of amplicons [5]. Visible bands on the test strips indicate positive reactions (Fig. 1). In this study, a CPA isothermal amplification and detection kit (Ustar Biotech, Hangzhou, China) was evaluated as whether it could accurately detect A. citrulli from pure bacterial culture and bacterial extracts from contaminated (or naturally infected) watermelon seeds.

A set of five primers were designed based on 16S rDNA of A. citrulli (GenBank AY702093.1). The five primer sequences are as follows: ACLF3: GGCTAATCCTGCCGACG, ACLB3: ACGCATTT CACTGCTACA, ACLBIP: CAGATGAAATCCCGGCTCGCCG TACTCCAGCGAT, ACDFSB1: BIOTIN-GCAAGCGTATACTCGAAATTACT, ACDFS5F2: FITC-CAACCTGGGAAGCTTGT.

Sensitivity of CPA assay on A. citrulli was determined by analyzing product produced from a ten-fold serial dilution of A. citrulli pure culture (Fig. 1(A)). Our results showed that the detection limit for the kit was about 3.7 × 10^3 CFU/ml per reaction (equivalent to 7.4 bacterium per reaction), which is similar to the sensitivity of normal PCR assay (Fig. 1(B)).

The specificity of the CPA assay was then evaluated by detecting 18 A. citrulli strains and 22 reference strains from the genus of Xanthomonas, Pseudomonas, Erwinia and Clavibacter. DNA of pure bacterial cultures was isolated using TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer’s instructions. Positive results were obtained for all A. citrulli strains, but not for all other non-Acidovorax strains. However, positive results were also obtained for Acidovorax avenae subsp. avenae and A. avenae subsp. cattleyae strains. Though the CPA assay could not differentiate closely-related Acidovorax strains from each other due to highly conserved 16S rDNA sequence, the CPA assay could still be used for detection of A. citrulli, considering that A. citrulli is only limited to plants in the Cucurbitaceae family, such as watermelon, melon, and cucumber; whereas other Acidovorax strains are not linked to these plant species. Combined with host specificity and pathogenicity, the CPA assay could be useful for detection of A. citrulli from watermelon seeds.

Next, 12 batches of watermelon seeds naturally infected with A. citrulli from fields and 5 batches of healthy watermelon seeds...
were tested by CPA and normal PCR. Seeds were suspended in sterile distilled water for 30 min and 1 ml leachate was centrifuged at 12,000 g for 10 min. DNA was extracted from precipitants using the DNA secure Plant Kit (Tiangen Biotech, Beijing, China). Positive results were obtained from all infected seeds, but not from healthy seeds, which were similar to the results from normal PCR, suggesting the CPA assay can be applied to detect bacteria from contaminated seeds.

CPA assay has been reported to detect *Mycobacterium tuberculosis* and *Enterobacter sakazakii* [6,7]. However, application of CPA in detecting plant pathogenic bacteria has not been reported. Here, we described the use of CPA kit for detection of *A. citrulli*. In this assay, DNA can be amplified by *Bst* DNA polymerase under isothermal conditions. The amplicons is detected in a simple, self-contained disposable DNA strip without opening the amplification tube, so post amplification contamination, which is associated with many nucleic acid amplification-based platforms, can be effectively eliminated. In the entire procedure, from sample preparation to visual readout, only a portable battery powered heater and small centrifuge are needed. Thus, the major advantages of this assay are its shorter turn-around time, no need of specific equipment, and easy to interpret results. So this assay can be used as an alternative to conventional PCR.

In summary, our results suggest that the CPA assay, combined the advantage of isothermal amplification with simple-to-perform and self-contained amplicons detection technology, is a valuable alternative to immunoassays and PCR-based tests for diagnosis of *A. citrulli*.

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### References


